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TETRAZOLE ANALOGUES OF AMINO ACIDS AND PEPTIDES—V[1]

SYNTHESES OF PEPTIDE DERIVATIVES CONTAINING TETRAZOLE ANALOGUES OF AMINO ACIDS IN C-TERMINAL POSITION

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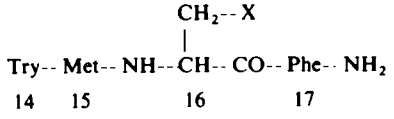
Abstract—The title compounds were prepared by coupling of N-protected amino acids with triethylammonium salts of tetrazole analogues of amino acids followed by removal of amino protection. Alternatively the products were prepared from amides of N-protected peptides by dehydration to the corresponding nitriles, formation of the tetrazole nucleus by the reaction of the cyano group with ammonium azide, and removal of amino protection.

IN 1969 Morley reported the synthesis of an analogue of C-terminal tetrapeptide amide sequence of gastrin wherein aspartic acid in position 16 was replaced by β-(5-tetrazolyl)-L-alanyl residue (1b).[2] This tetrazole analogue of the physiologically active tetrapeptide amide was found to be at least as potent as its aspartyl relative. All the other synthetic analogues derived by change at the Asp position were devoid of activity.[3,4] It is known from McManus and Herbst work that the acidity of the tetrazole group corresponds closely with that of the carboxyl group.[5] Therefore, it was concluded that 5-tetrazolyl residue can replace Asp β-carboxyl group in proton transfer reaction, essential for gastrin activity.[3]



1a: X = --COOH (C-terminal sequence of gastrin)

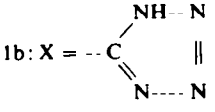


FIG 1

Since dimensions and even shape as well as a degree of proton availability (acidity) of the 5-tetrazolyl group are similar with those of the carboxyl group,[2,3,5] replacement of C-terminal amino acid residue in biologically active peptides for its tetrazole analogue would create an interesting class of compounds for structure-function relationship studies. The aim of the present paper is to report two general routes for the synthesis of these compounds.

The first route is shown in Fig. 2. The synthesis started with amides of N-protected peptides. As exemplified in Fig. 2 dehydration of the amide of N-benzyloxycarbonyl-glycylglycine (2) with phosphoryl chloride in pyridine[6] gave the corresponding nitrile (3). Alternatively the nitrile (3) was prepared by coupling of the *p*-nitrophenyl ester of N-benzyloxycarbonyl-glycine (5) with aminoacetonitrile hydrogensulphate[7] in the presence of triethylamine. The next step involved the formation of tetrazole nucleus (4)* from the intermediate nitrile by the action of ammonium chloride and sodium azide in warm (95–100°) dimethylformamide.[8] The results of preparation of intermediate nitriles are presented in Table 1.

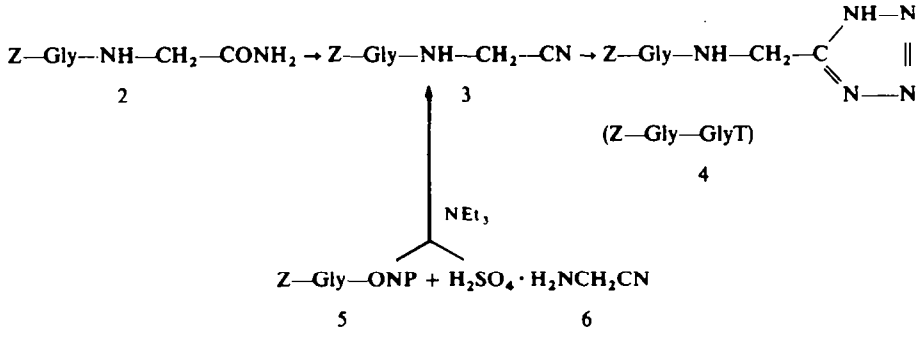


FIG 2

TABLE 1. NITRILES OF N-PROTECTED DIPEPTIDES

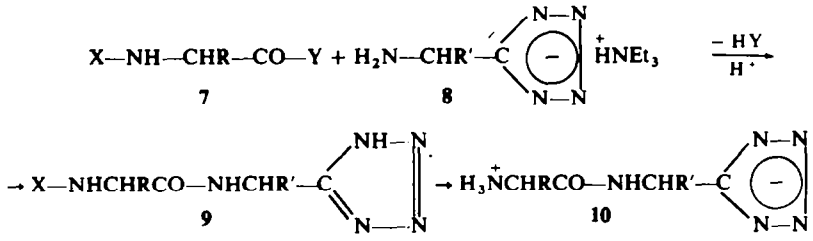
No	Compound	Method*	Yield %	M.p. °C	Formula	%N calc.	%N found
1.	Z-DL-Ala-$NHCH_2CN$	A	51	123–125	$C_{13}H_{15}N_3O_3$	16·09	16·30
2.	Z-β-Ala-$NHCH_2CN$	A	71	106–108	$C_{13}H_{15}N_3O_3$	16·09	16·31
3.	Z-Gly-$NHCH_2CN$	A	64	148–150	$C_{12}H_{13}N_3O_3$	17·00	16·93
		B	63				
4.	Z-DL-Phe-$NHCH_2CN$	A	66	125–127	$C_{19}H_{19}N_3O_3$	12·46	12·30

* A—coupling of p-nitrophenyl or 2,4,5-trichlorophenyl ester of N-benzyloxycarbonyl amino acid with aminoacetonitrile;
B—from amide of N-benzyloxycarbonyl peptide

The second synthetic route is shown in Fig. 3. This procedure involved condensation of N-protected amino acid with triethylammonium salt of tetrazole analogue of amino acid.[10] Couplings were performed by means of active 2,4,5-trichlorophenyl esters,[11] *p*-nitrophenyl esters,[12] mixed anhydrides,[13–15] and acid chlorides.[16, 17] Morley reported the synthesis of tetrazole analogue of N-benzyloxycarbonyl-β-benzyl-L-aspartyl-L-phenylalanine prepared by aminolysis of the active 2,4,5-tri-

* Proposal of nomenclature and symbolism of tetrazole analogues of amino acids is given in Ref. 9 (see also Part IV—This Journal)

chlorophenyl ester.[2] Under Morley's conditions we obtained only 44% yield from coupling of the 2,4,5-trichlorophenyl ester of N-benzyloxycarbonyl-DL-phenylalanine with tetrazole analogue of glycine (glycinetetrazole). The results of aminolysis were, however, considerably improved (90% yield) by carrying out the reaction in the presence of a small amount of water (Table 2). On the other hand only low yield was obtained from the reaction of active cyanomethyl ester[18] with glycinetetrazole.



X = t-butyloxycarbonyl (BOC), phthaloyl (PHT) or benzyloxycarbonyl (Z)
Y = nitrophenyl (NP), 2,4,5-trichlorophenyl (CP), —OCOOEt or —Cl

FIG 3

TABLE 2. RESULTS OF AMINOLYSIS OF ACTIVE ESTERS OF N-BENZYLOXYCARBONYL-DL-PHENYLALANINE WITH TRIETHYLAMMONIUM SALT OF GLYCINETETRAZOLE (TETRAZOLE ANALOGUE OF GLYCINE)

No	Ester	Solvent*	Yield %
1.	Z-DL-Phe-OCP	DMF (2 ml)	44
2.	Z-DL-Phe-OCP	DMF (2 ml) + water (0·3 ml)	89
3.	Z-DL-Phe-ONP	DMF (2 ml) + water (0·3 ml)	91
4.	Z-DL-Phe-OCH_2CN	DMF (2 ml) + water (0·3 ml)	10

* To 1.1 mmole of active ester in DMF and water 1 mmole of GlyT was added followed by 1 mmole of NEt_3

The results of preparation of tetrazole analogues of N-protected peptides are summarized in Table 3. In general the best route to this class of peptide derivatives is "via" the aminolysis of active 2,4,5-trichlorophenyl esters or *p*-nitrophenyl esters with triethylammonium salts of aminoalkyltetrazoles.

Some of the N-benzyloxycarbonyl derivatives were converted into free analogues of peptides (10) by treatment with hydrobromic acid in acetic acid followed by neutralization of hydrobromide with lithium hydroxide in aqueous methanol. The results are summarized in Table 4.

Inspection of IR spectra revealed that tetrazole analogues of peptides like peptides themselves exist in zwitterion structure.[19]

TABLE 3. N-PROTECTED PEPTIDE DERIVATIVES CONTAINING TETRAZOLE ANALOGUES OF AMINO ACIDS IN C-TERMINAL POSITION

No	Compound	Method of synthesis[a]	Yield %	M.p. °C	Formula	% N calc.	% N found
1.	BOC-L-Met-GlyT[e]	C	79	161–162[b]	$C_{12}H_{22}N_6O_3S$	25·44	25·38
2.	PHT-DL-Ala-GlyT	B	33	224–226[c]	$C_{13}H_{12}N_6O_3$	28·00	28·33
		C	73				
		D	65				
3.	PHT-DL-Phe-GlyT	B	68	260–262[c]	$C_{19}H_{16}N_6O_3$	22·34	22·60
		C	91				
		D	76				
4.	Z-DL-Ala-GlyT	A	58	167–169[b]	$C_{13}H_{16}N_6O_3$	27·62	27·48
		B	62				
		C	81				
5.	Z-β-Ala-GlyT	A	77	179–181[d]	$C_{13}H_{16}N_6O_3$	27·62	27·20
6.	Z-Gly-GlyT	A	80	188–190[c]	$C_{12}H_{14}N_6O_3$	28·95	28·54
		B	47				
		C	91				
7.	Z-Gly-DL-PheT	C	73	69–71[b]	$C_{19}H_{20}N_6O_3$	22·10	22·25
8.	Z-Gly-DL-ValT	C	58	125–127[b]	$C_{15}H_{20}N_6O_3$	25·29	25·63
9.	Z-DL-Phe-GlyT	A	85	208–209[b]	$C_{19}H_{20}N_6O_3$	22·10	21·72
		B	66				
10.	Z-L-Phe-GlyT[f]	C	89	212–214[b]	$C_{19}H_{20}N_6O_3$	22·10	22·04
11.	Z-DL-Phe-Gly-GlyT[i]	C	92	201–203[c]	$C_{21}H_{23}N_7O_4$	22·42	22·47
12.	Z-L-Try-GlyT[g]	C	98	203–204[c]	$C_{21}H_{21}N_7O_3$	23·38	23·26
13.	Z-L-Val-GlyT[h]	C	91	215–217[c]	$C_{15}H_{20}N_6O_3$	25·29	24·95

[a] From nitrile of N-protected peptide (A); from couplings of N-protected amino acid with triethylammonium salts of tetrazole derivatives by means of mixed anhydrides (B), p-nitrophenyl or 2.4.5-trichlorophenyl esters (C) or acid chlorides (D).

[b] Recrystallized from methanol-water.

[c] Recrystallized from methanol.

[d] Recrystallized from water.

[e] $[\alpha]_D^{20} - 12°$ (c = 1, DMF).

[f] $[\alpha]_D^{20} - 24°$ (c = 1, DMF)

[g] $[\alpha]_D^{20} - 34°$ (c = 1, DMF).

[h] $[\alpha]_D^{20} + 6°$ (c = 1, DMF).

[i] From coupling of p-nitrophenyl ester of N-benzyloxycarbonyl-DL-phenylalanine with triethylammonium salt of glycyl-glycinetetrazole.

TABLE 4. FREE PEPTIDE DERIVATIVES CONTAINING TETRAZOLE ANALOGUE OF AMINO ACID IN C-TERMINAL POSITION

No	Compound	Yield %	M.p. °C	Formula	% N calc.	% N found
1.	DL-Ala-GlyT	84	236–237 dec.	$C_5H_{10}N_6O$	49·41	49·56
2.	β-Ala-GlyT	94	237–238 dec.	$C_5H_{10}N_6O$	49·41	49·81
3.	Gly-GlyT	73	232–233 dec.	$C_4H_8N_6O$	53·85	53·26
4.	DL-Phe-GlyT	72	239–241 dec.	$C_{11}H_{14}N_6O$	34·14	33·98
5.	DL-Phe-Gly-GlyT	94	281–282 dec.	$C_{13}H_{17}N_7O_2$	32·33	32·11
6.	L-Val-GlyT	75	235–237 dec.	$C_7H_{14}N_6O$	42·41	42·83

EXPERIMENTAL

M.ps are uncorrected.

Nitriles of N-protected dipeptides (*Table* 1)

(a) *From active esters of* N-*protected amino acids*. *p*-Nitrophenyl or 2,4,5-trichlorophenyl ester of N-protected amino acid (10 mmoles) in dioxan (20 ml) was added to a stirred solution of aminoacetonitrile hydrogensulphate (1·93 g—12·5 mmoles) in water (2 ml). After addition of triethylamine (3·5 ml–25 mmoles) the mixture was stirred for further 18 hrs and then the solvent was evaporated under reduced pressure. The residue was dissolved in AcOEt (20 ml) and washed with 1 N hydrochloric acid, $NaHCO_3$ solution, water and then dried over $MgSO_4$. The crude nitrile was obtained after evaporation of AcOEt under reduced pressure. The products were recrystallized from methanol-water.

(b) *From amides of N-protected dipeptides*. Phosphoryl chloride (1·25 ml) in dry methylene dichloride (2 ml) was added during 5 min. to cooled to $-5°$ and stirred solution (or finally ground suspension) of amide of N-protected dipeptide (10 mmoles) in dry pyridine (10 ml). The resulting mixture was stirred at $-5°$ for 1 hr. and then the nitrile was separated by addition of ice–water (50 ml). The solid was collected and washed with water.

N-protected peptide derivatives containing tetrazole analogues of amino acids in C-terminal position (*Table* 3)

(a) *From nitriles of N-protected dipeptides*. A suspension of the nitrile of N-protected dipeptide (10 mmoles), sodium azide (0·72 g—10·4 mmoles) and ammonium chloride (0·59 g—11 mmoles) in dimethylformamide (8 ml) was heated to 95–100° for 16–24 hrs. and then the solvent was evaporated under reduced pressure. To the residue 1 N hydrochloric acid (15–20 ml) was added (caution: HN_3 evolved) and then the solid was collected, washed with cold water and recrystallized from appropriate solvents (Table 3).

(b) *From couplings of N-protected amino acids with triethylammonium salts of tetrazole derivatives by*: *Mixed anhydride*. N-protected amino acid (10 mmoles) and triethylamine (1·4 ml—10 mmoles) were dissolved in dry chloroform (15 ml). To the solution cooled to -5 ethyl chloroformate (0·98 ml—10 mmoles) was added portionwise with stirring. After 7–8 min. the tetrazole analogue of amino acid (11 mmoles) and triethylamine (1·54 ml—11 mmoles) in DMF (15 ml) were added and the mixture was vigorously stirred for half an hour at 0° and for further 3 hr at room temperature. After evaporation of the solvent under reduced pressure the residue was treated with 1 N hydrochloric acid (15–20 ml). The separated product was filtered and washed several times with cold water.

Active esters

A suspension of *p*-nitrophenyl or 2,4,5-trichlorophenyl ester of N-protected amino acid (11 mmoles), tetrazole analogue of amino acid (10 mmoles) and triethylamine (1·4 ml—10 mmoles) in DMF (20 ml) and water (3 ml) was stirred for 3 hr. (after about 15–20 min. a clear solution resulted). Then the $NaHCO_3$ solution was added and the content of the flask was extracted three times with AcOEt (3 × 15 ml). The aqueous layer was acidified with 1 N hydrochloric acid and the separated crude product was collected and washed with cold water.

Acid chlorides

To a stirred solution of acid chloride of N-phthaloyl amino acid (11 mmoles) in dry THF (22 ml) the tetrazole analogue of amino acid (10 mmoles) and triethylamine (2·94 ml—21 mmoles) in dry THF (20 ml) were added. Stirring was continued for 3 hr and then the solvent was evaporated under reduced pressure. To the residue 1 N hydrochloric acid (15–20 ml) was added and the separated solid was then collected, washed with cold water and recrystallized from appropriate solvent.

Removal of amino protection. Free tetrazole analogues of peptides (*Table* 4)

The analogue of N-benzyloxycarbonyl peptide (2 mmoles) was treated with hydrobromic acid in acetic acid (2 ml of 6 N solution). After 45 min. dry ethyl ether was added; the separated hydrobromide of tetrazole derivative was collected and washed several times with ethyl ether. The dry hydrobromide was dissolved in methanol, treated with an equimolar amount of aqueous lithium hydroxide and left overnight in refrigerator. On the next day the crystalline free analogue of peptide was filtered and washed with a minimal amount of methanol. The products were recrystallized from aqueous methanol.

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